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SYNOPSIS ON

"PHYTOCHEMICAL INVESTIGATION OF VERBENACEOUS PLANTS FOR CONTROL OF POST HARVEST DISEASES OF FRUITS IN NANDED."

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"PHYTOCHEMICAL INVESTIGATION OF VERBENACEOUS PLANTS FOR CONTROL OF POST HARVEST DISEASES OF FRUITS IN NANDED."

INTRODUCTION:

Phytochemicals are the naturally occurring compounds produced as a result of primary, secondary metabolism in the plant body (Harborne, *et al.,* 1999) which exhibit biological activity, helps in plant growth by providing an essential defense mechanism against various biotic and abiotic factors (Molyneux, *et al,.* 2007). For study of phytochemicals a researcher need to first go for the extraction, isolation of compounds from the particular plant origin, then need to define the structure and to test with in vitro, or in vivo examination. A single plant may contain various phytochemicals (Ahn, 2017). Phytochemistry also relies on ethno botany for knowing pharmacologically active substances in a particular plant of a region, leading to discovery of several medicinally important compounds. Medicinal plants are easily available in surrounding region and cheaper than present day chemical medicines. Now day's phytochemical properties of plant species can be explored for the management of plant and animal diseases. Research in this direction is currently going all over the world.

Postharvest decays of fruits and vegetables account for significant levels of postharvest losses. It is estimated that about 20–25% of the harvested fruits and vegetables are decayed by pathogens during postharvest handling even in developed countries. In developing countries, postharvest losses are often more severe due to inadequate storage and transportation facilities. Synthetic fungicides are primarily used to control postharvest diseases of fruits and vegetables. However, the global trend appears to be shifting towards reduced use of fungicides and hence, there is a strong public and scientific desire to seek safer and eco-friendly alternatives for reducing the decay loss in the harvested commodities (Sharma, *et al.,* 2009).

Along with the economic losses the diseased fruits also contain serious pathogens responsible for causing several health issues. Fungi such as *Penicillium sp., Alternaria sp., Fusarium sp.* produces mycotoxins under certain conditions (Legan, 2000).

In India the Verbenaceae family has 23 genera (Hooker 1885), 21 genera and 125 species (Singh and Jain 1974), found in South India, West part of India and in Himalayas.

The plants exhibit variety of habit, majority are shrubs, climbers. Common Flora belonging to Verbenaceae family in the Marathwada region as studied by V. N. Naik (1998) includes *Citharexylum spinosum ,Clerodendrum calamitosum ,Clerodendrum inerme, Clerodendrum multiflorum, Clerodendrum philippinum, Clerodendrum serratum, Clerodendrum erecta, Duranta erecta, Gmelina arborea, Gmelina asiatica, Gmelina philippinesis, Holmskioldia sanguine, Lantana camara, Lantana indica, Petrea volubilis ,Phyla nodiflora, Priva cordifolia, Stachytarpheta jamaicensis ,Tectona grandis, Verbena bipinnatifida, Verbena officinalis, and Vitex negundo.*

REVIEW OF LITERATURE (Previous work done in the relevant area)

Chemical fungicides provide the primary means for controlling postharvest fungal decay of fruit and vegetables. Continuous use of fungicides has faced two major obstacles increasing public concern regarding contamination of perishables with fungicidal residues, and proliferation of resistance in the pathogen population. The ultimate aim of recent research in this area has been the development and evaluation of various alternative control strategies to reduce dependency on synthetic fungicides. Several non-chemical treatments have been proposed for fungal decay control. Recently, the exploitation of natural products to control decay and prolong storage life of perishables has received more and more attention. Biologically active natural products have the potential to replace synthetic fungicide (Pramila and Dubey, 2004)

A considerable amount of work has been carried out all over the world on the effect of plant extracts on the quality and shelf life in various fruit and vegetable crops. Fungal infections are one of the major causes of post harvest rots of fresh fruits. They cause significant economic losses in the commercialization phase, and are rendered unfit for human consumption. Neem (*Azadirachta indica* A. Juss) and chinaberry (*Melia azadrach*) are two trees belonging to the mahogany family (Meliaceae). The leaf extracts of both the plants were evaluated against two tomato pathogenic fungi *Alternaria solani* and *Fusarium oxysporium*, the causal agents of early blight and wilt disease of tomato plant. The aqueous and alcoholic leaf extracts of different concentration (5%, 10%, 15% and 20%) were tested against *Alternaria solani* and *Fusarium oxysporium.* The results showed that, the extracts significantly inhibited the mycelial growth of the fungal

pathogens at the different concentrations tested and the rate of inhibition differed from one extract to the other. Plants contain phenolic substances and essential oils, which are inhibitory to micro-organisms. The presence of these compounds in these extracts has been reported to be responsible for their antifungal properties which control various pests including fungi (Borathkar, *et al*. 2002).

Korsten and Obagwu (2003) studied effect of water and ethanol extracts of garlic cloves against *Penicillium digitatum* and *P. italicum*, responsible for green and blue mold disease in Citrus. Extracts were tested either alone, or in combination with vegetable (sunflower) cooking oil or fruit wax at the rate of (0.1% v/v), using two orange cultivars (Valencia and Shamouti), and grapefruit. Treated fruits were stored at 10 ± 1^{o} C, and 90–95% relative humidity for 30 days. All concentrations of extracts were more effective than the water control in inhibiting the growth and development of both pathogens, but were not as effective as the fungicide treatment. A remarkable increase in the activity of garlic extracts was observed when extracts were mixed with oil. Consequently, the treatment comprising 1% extract plus oil was as effective (100% control) as the fungicide treatment in controlling both green and blue molds on Valencia oranges.

The aqueous and organic solvent extracts of garlic contained very potent fungicidal and bactericidal activity against a variety of plant pathogens. They were able to control post-harvest brown rot of peaches effectively by dipping the fruit in an odorless garlic extract. A number of essential oils are produced by plants that have fungicidal activity and may have potential for the control of post-harvest diseases of fruit. Homeopathic drugs of plant origin have also been shown to control certain post-harvest diseases (Wilson, *et. al.*,1991).

The study made by Bhutia (2016) on one of the common plant of Verbenaceae, the *Clerodendrum inerme* reported that the leaf extracts show significant growth inhibition of *Collectotrichum musae*, post-harvest pathogen of Banana.

Bhardwaj, *et. al.,* (2002) studied the effect of different concentrations of neem leaf extracts on the storage quality of mandarin (*Citrus reticulata*) Nagpur Santra and reported that among various treatments used neem leaf extract (20%) was significantly better in retaining higher ascorbic acid content (27.17 mg/100 ml. of juice) as compared

to control fruits where it was only after storage. He found that aqueous extracts from different plants can be an alternative to chemicals in controlling post-harvest pathogens as they are environmentally safe strategies for controlling the post- harvest storage pathogens in different crops.

Plant extracts are being used to manufacture natural or bio insecticides, which are environmental friendly and do not have any toxic effects on plants and soil. Moreover they possess fungicidal and insecticidal properties. So these can be used as compare to synthetic/ chemical products. Various extracts such as neem leaf extract castor oil and neem oil on citrus fruits and reported that, among these extracts neem was best in retaining most of biochemical characteristics such as TSS, acidity, pectin and ascorbic acid content. Moreover the extract of neem, marigold and other plant extracts increase the shelf life of the fruits and vegetables by reducing the fungal and bacterial spoilage (Borathkar, *et.al.,* 2002).

The literature showed that the plants belonging to the Verbenaceae have little exposure for the control of post harvest diseases therefore we have planned to work in this direction. Though verbenaceous plants shows potential antimicrobial, antioxidant and other biological activities, yet we find scope for further explorations of plants from the local forest.

In Nanded region farmers cultivate different fruits of economic importance like: Mango, Guava, Papaya, Pomegranate, Citrus, Chiku, Banana, etc. The undertaken research may become useful in controlling different post harvest diseases of fruits in this region.

OBJECTIVES OF RESEARCH/ PROPOSED HYPOTHESIS:

Following Objectives are proposed for present research work:

1. Survey of Verbenaceous flora from local forest.
2. Phytochemical analysis of some selected plants of family Verbenaceae.
3. To perform HPTLC profiling of selected plants.
4. To determine antimicrobial potential of plant extract against post harvest pathogen.
5. To develop strategies to prevent and control post harvest diseases of fruits.
6. To develop strategies to enhance the shelf life of fruits.

METHODOLOGY TO BE ADOPTED:

Phase-I: Literature Survey, Collection of Diseased fruits.

This phase of study will emphasize on literature survey, collection of diseased fruits and isolation of pathogens from fruits. The isolation of pathogen will be done using standard protocols. The experiments will be set up to prove pathogenicity of isolated pathogen.

Phase-II: Plant collection and phytochemical analysis.

This phase of study will emphasize on Verbenaceous plant collection from local forests. Plant identification will be done using standard floras (Naik V. N. 1998). Plant extraction and phytochemical and the phytochemical analysis will be done from different plant parts. For phytochemical analysis standard procedures as mentioned by Harborne, 1973; Kokate *et al.*,1998; Sadasivan & Manikam, 2005; Thimmaiah, 1999 will be followed.

Phase-III: HPTLC Analysis of Plants.

HPTLC analysis of selected plant extracts will be done in central instrumentation facility available in college. HPTLC will be done by standard protocols (Mona , *et.al.* 2012).

Phase-IV Antimicrobial activities of Plants extracts.

This phase of study will emphasize on revealing the potential of plant extract for antimicrobial properties against the isolated microorganisms. Antimicrobial activities will be tested by the standardized methods.

Phase-V: Effect of plant extracts on fruits.

This phase of study will emphasize on giving treatment of plant extracts to different fruits and studying its effect on growth of post harvest pathogens and the shelf life of fruits.

Phase-VI: Thesis writing and Submission.

Scientific writing of thesis will be done for final submission to University.

IMPORTANCE OF STUDY/SOCIETY APPLICATION:

International status:

Various researchers have contributed their efforts in revealing the potential of phytochemicals. At International level efforts are being done to develop an eco friendly approach for control of post harvest diseases by using plant extracts.

Recent years, in China many scholars have selected a large number of extracts from herbal medicines that inhibit the activity of *P. digitatum* and *P. italicum*. The effect of rosemary (*Rosmarinus officinalis L.*) extracts on the growth of the green mold of

Citrus was studied by Hendel *et.al.* (2016) and in vitro antifungal assays showed a clear inhibitory effect of the rosemary essential oils and methanol extract on the growth of *P. digitatum*. An aqueous crude extract prepared from lyophilized leaves of *Solanum nigrum* showed an inhibitory effect on *P. digitatum* with a remarkable inhibition zone. *Solanum nigrum* extracts showed an important preventive antifungal effect where 100% of inhibition was observed at 7 days of storage. Previous studies regarding antifungal effect of ethanolic extracts of Ficushirta Vahl's fruits on *P. italicum* and antifungal assays revealed the presence of a major flavonoid pinocembrin-7-O-β-D-glucoside as a strong antifungal agent. In another study which uses ethyl acetate extracts of *Ramulus cinnamomi* plant showed resistance against several Citrus pathogens and good antifungal properties against *P. digitatum* and *P. italicum*

The research work conducted by Mondal *et al.,* (2011) in Bangladesh showed that, there was significant variation among the treatments in respect of shelf life, disease incidence and severity of papaya. The shelf life increased due to different postharvest treatments over control. The results of the experiment revealed that garlic extract gave the longest shelf life (16.00 days) followed by bishkanthali extract (15.00 days) and the shortest shelf life was recorded under control (8.67 days). Statistically, identical results were given by Datura and Onion extracts. From experimental results it was recommended that garlic extract would be a promising tool for extending shelf life of papaya.

National status:

India is a country with a lot of Biodiversity, in regard to the work done by several naturalists there is still a need to explore the bio diversity, the country is blessed by a great diversity of plants that are used at every step of human life, not only as food, cloth ,timber, there are various fields that can be explored to further study of plants. Phytochemical analysis is one of the important studies that can help us at various path of life. Phytochemicals are important for us in various ways such as they are good source of medicines also.

In regard to the national status of the study related to the Phytochemical analysis several Indian researchers have made immense contribution in this field from long time, But in concern to the work done in recent years efforts of few of them are mentioned in this synopsis. In India, there are various reports in regarding the phytochemical analysis

of several plants belonging to the Verbenaceae, The work done by Shetty and Patil (2015) revealed that the *V. negundo* seed extract exhibited antioxidant activity comparable with ascorbic acid. According to the investigation done by P. Tripathi, *et al.,(*2017), plants of Verbenaceae, are potent source of diverse biologically active phytochemicals. Methanol, petroleum ether and aqueous extracts revealed the presence of various secondary metabolites such as phenolic compounds, flavonoids alkaloids, carbohydrates, proteins, saponins, steroids, tannins, starch, glycosides, resins, anthocyanins and terpenoids in varying amounts.

Nabakishor Nongmaithem, (2014) reported neem oil as effective measure to control postharvest diseases of Guava caused by *Pestalosia psidii* , *Gloesporium psidii* ,*Rhizoctonia solani* , *Fusarium sp., Alternaria alternata* , and *Geotrichum candidum.*

According to Panhwar (2006), the essential oil extracts of red thyme (*Thymus zygis*), clove buds (*Eugenia caryophyllata*) and cinnamon leaf (*Cinnamomum zeylanicum*) can be used to prevent the growth of *Botrytis cinerea.* He also reported effectiveness of oil extracts of *Monarda citrodora* and *Melaleuca alternifolia* against several fungi responsible for post harvest diseases.

Dixit *et al.* (1995) reported that vapors produced by using leaf extracts of *Ageratum conyzoides* exhibit strong toxic action against *Penicillium italicum* causing blue mould rot of mandarins oranges.

Kanan and Ghassan (2008) studied in vitro antifungal activities of various plant crude extracts and fractions against citrus post harvest disease agent *Penicillium digitatum*. Crude extracts of several plant materials (fenugreek seeds, harmal seeds, garlic cloves, cinnamon bark, sticky fleabane leaves and nightshade leaves and seeds) are tested. Concerning fraction of garlic extract, only methanolic fraction has resulted in complete inhibition of fungal growth.

Significance of the study:

In context to the studies on phytochemical analysis there is increasing need to explore more about the sources of the compounds of natural originthat can be isolated from our surrounding, because the phytochemicals are abundant in our nature but still a lot of

studies are to be done. The phytochemicals show significant roles in different aspects of human life like: medicines, alternative sources of chemical pesticides, antioxidant activities, etc. In future there might generate an opportunity to farmers to grow the plants which are important in relation to the phytochemicals which may generate very good revenue as compare to the traditional plants. The Verbenaceae family in our region needs to be explored in regard to the investigation of various phytochemicals, and a lot of work is still to be done in this area of study. From several reports it is also found that the members of Verbenaceae are good source of phytochemicals that have potential anti microbial activities which may further be used to control the post harvest diseases of fruits in our region. As the study is in regard of the Verbenaceae family it may also be possible to reveal various plants belonging to this family which are of high importance but unnoticed from the world, it will also add information regarding the ethno botanical aspects of this family.

PROPOSED WORK PLAN (Year wise plan of work and targets to be achieved):

First year:

1. Collection and Survey of diseased fruits from local region.
2. Isolation of pathogen from diseased fruits
3. Proving Pathogenicity.

Second year:

1. Survey of Verbenaceous plants from local forest.
2. Phytochemical analysis of selected plants
3. HPTLC analysis

Third year:

1. Antimicrobial activity of plant extract.
2. Effect of plant extracts for control of post harvest diseases of fruits
3. Thesis writing and submission to the University.

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